Microarray-Based Functional Analysis of Metal-Reducing and Radiation-Resistant Bacteria

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Posters, 131, 134

Challenges and approaches

- Challenegs are to understand gene functions and regulatory network
  - 30-60% open reading frames are functionally unknown.
  - Functional assignment by bioinformatic approaches may be misleading or incorrect due to complicated evolutionary processes, needs to be experimentally confirmed.
- Integrated experimental approach is the solution.
  - Microarrays: Gene expression differentiation by whole genome microarrays
  - Genetic tools: Genetic mutant generation and characterization by microarrays and mass spectrometry
  - Biochemical tools: Protein purification and characterization by mass spectrometry, 2D gels, X-rays, phage-display and etc.

Shewanella oneidensis MR-1

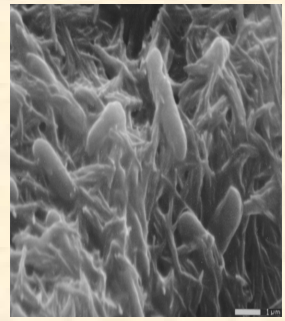
(formerly *S. putrefaciens,* Venkateswaran, et al. 1999)

Habitats:

- lake & marine sediments
- deep sea
- oil brine
- spoiled food

Respiratory Versatility (e- acceptors):

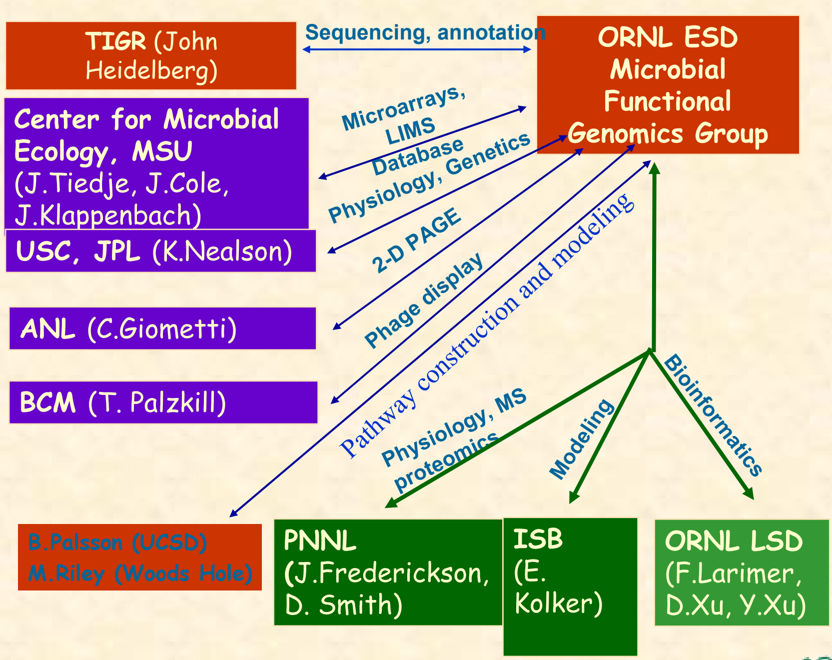
- oxygen
- glycine, fumarate, DMSO, TMAO
- nitrate, nitrite,thiosulfate, sulfite
- Fe and Mn oxides/oxyhydroxides



Shewanella on magnetite crystals
Photo courtesy of K.H.Nealson

Poster number: 134

DOE Shewanella Federation



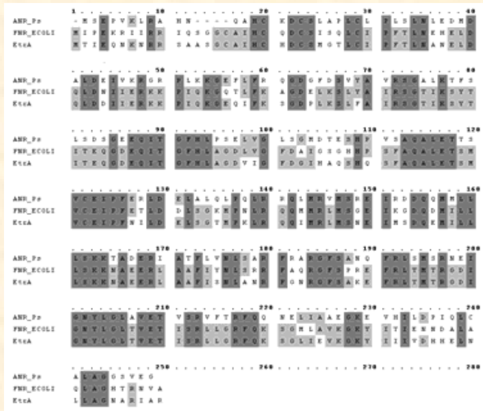
Roles of ORNL Team in Shewanella Federation

- Point of Contact: Jim Frederickson, PNNL
- Central web site: www.shewanella.org
- Roles of ORNL team
  - Microarray-based gene expression analysis
  - Genome-wide mutagenesis and target mutagensis
  - Phage-display for studying protein-ligand interactions
  - Sequence annotation

Two examples in using microarrays for functional analysis

- Hypothesis-driven: e.g., mutant characterization:
  - etrA mutant of Shewanella oneidensis
- Exploratory: Gene profiling under different conditions:
  - Responses of Deinococcus radiodurans to acute radiation.

Comparative sequence analysis of the putative *etrA* gene from *S. oneidensis* MR-1

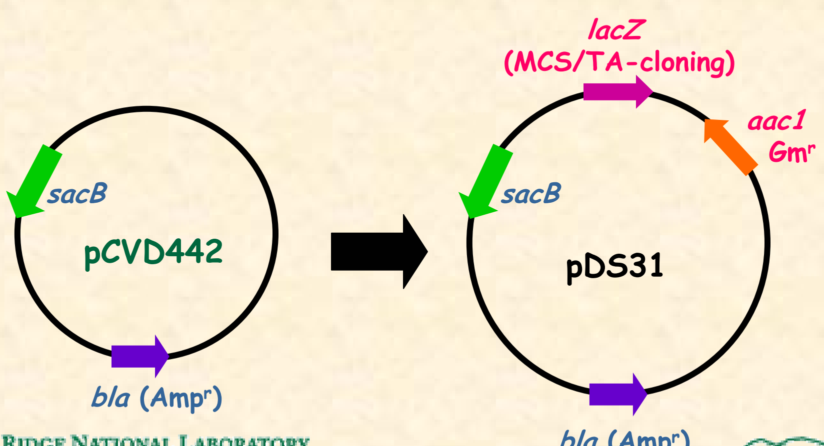


S. oneidensis MR-1 *etrA:*

- 73.6% identity to *E. coli fnr* gene product, Anaerobic metabolism
- 50.8% identity to *P. fluorescens anr* gene product(AAC38593)

Development of genetic vectors for in-frame deletion

- Vector modification (R6K suicide vector pCVD442 carrying *sacB)*



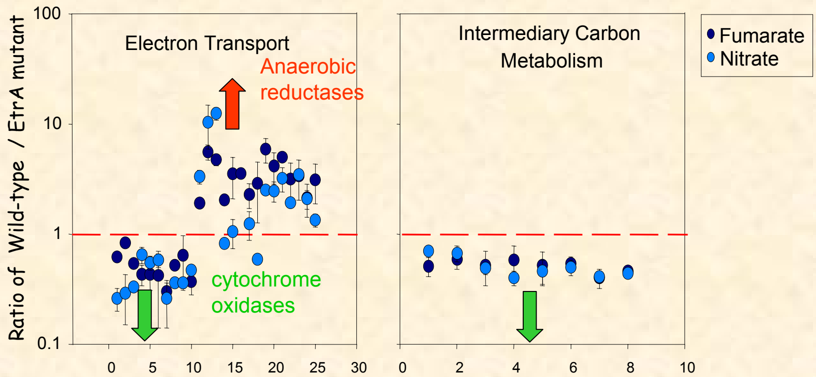
Phenotype analysis of an electron transport regulator gene, *etrA*

	MR-1 (wild-type)	$EtrA^-$	
Growth and/or reduction of:			
MnO_2	+	+	
$Fe(OH)_3$	+	+	
Fe(III) citrate	+	+	~30% decrease in the initial growth rate
Nitrate	+	+/-	
Nitrite	+	+/-	
DMSO	+	+	
TMAO	+	+	
Fumarate	+	+/-	~40% decrease in the initial growth rate
Thiosulfate	+	+	
Sulfite	+	+	
AQDS	+	+	

Hypotheses

- The *etrA* gene is not functional, i.e, a pseudogene;
- The *etrA* gene is functional, but not involved in anaerobic energy metabolism;
- The *etrA* gene is involved in anaerobic energy metabolism, but there are other dissimilar genes in *S. oneidensis* MR-1 that encode proteins with similar functions to that of *Etr*A.
- The *etr*A gene has the same function as the *fnr* in *E. coli*, but there are genes functionally similar to the *etr*A regulated genes and they are not controlled by *Etr*A.

Energy metabolism genes (33 genes)



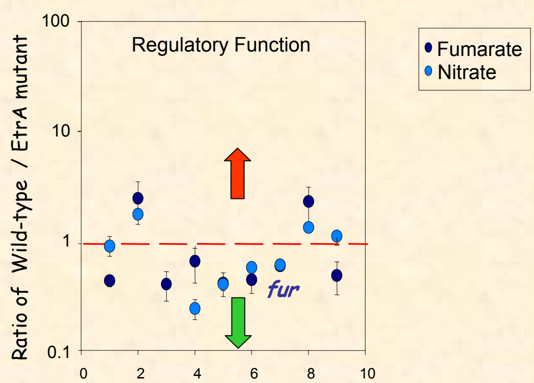
Induced by *etrA*:

- NO_3(*napBGHA*), prismane
- NO_2 (*nrfA*) reductases
- fumarate reductases (*frdABC*, *fcc*)
- DMSO reductase (*dmsAB*)
- formate DH (*fdhAB*),
- Ni/Fe hydrogenase (*hydABC*)

Repressed by *etrA*:

- cytochrome *c*- , *cbb*$_3$-,and *d*-oxidases
- NADH dehydrogenase (*ndh)*
- citrate synthase (*gltA)*
- succinyl-CoA synthetase (*sucCD*)
- α-ketoglutarate DH (*sucAB*)
- succinate DH (*sdhAB*)

Regulatory genes (9 genes)



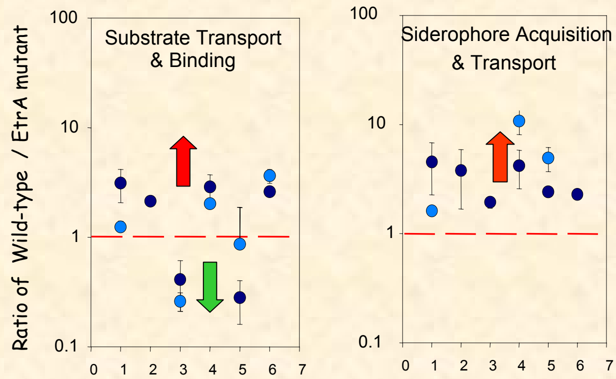
Induced by *etrA*:

- chemotaxis CheV homolog
- tetrathionate sensor kinase (*ttrS*)

Repressed by *etrA*:

- ferric uptake regulator (*fur*)
- histidine utilization repressor (*hut)*
- transcriptional regulator, DeoR family
- transcriptional regulator, LysR family
- transcriptional regulator, LacI family

Transport genes (12 genes)



Induced by *etrA*:
- copper ABC transporter (*nosF*)
- sulfate ABC transporter (*cysW*)
- siderophore acquisition genes

Repressed by *etrA*:
- lactate permease (*yghK*)
- ABC transporter

Putative FNR-binding motifs upstream of *S. oneidensis* genes affected by *etrA* mutation

E. coli fnr mutant is complemented by *etrA*:

- **FNR and EtrA binding sequences are very similar**

  TTGAT N_4 ATCAA

- **27 operons of *S. oneidensis* affected by the *etrA* disruption contained putative FNR motifs**

  NO_3, NO_2, DMSO, and fumarate reductases
  prismane, formate DH, Ni/Fe hydrogenase
  cytochrome *c*- and *d*-oxidases, NADH dehydrogenase
  ferric uptake regulator

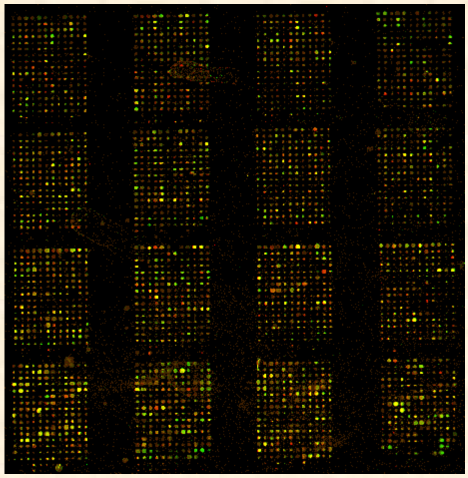
Microarray profiling & DNA-binding motif searching suggest that EtrA is involved in global gene regulation:

- EtrA participates in the regulation of genes involved energy metabolism, regulation, transport and biosynthesis
- EtrA has an indirect effect on gene expression by controlling the transcription of some regulatory genes such as *fur*
- EtrA acts in concert with other regulatory proteins, such as ArcA, CRP, and NarL to control the transition from aerobic to anaerobic conditions

Whole genome microarrays

- **Shewanella oneidensis MR-1:** **Metal-reducing bacterium**
- **Deinococcus radiodurans R1:** **Radiation-resistant bacterium (Mike Daly, poster 123)**
- **Rhodopseudomonas palustris:** **Photosynthetic bacterium (Caroline Harwood, Bob Tabita; posters: 127; 128)**
- **Nitrosomonas europaea:** **Ammonium-oxidizing bacterium (Dan Arp; poster 136)**

Deinococcus radiodurancs R1 whole genome microarrays



- Total ORFs: 3186
- No primer identified: 72
- Genes with >75%: 69
- Genes with primers designed 42
- No amplification: 42
- Genes on slide: 3003
- Genome coverage: **95%**

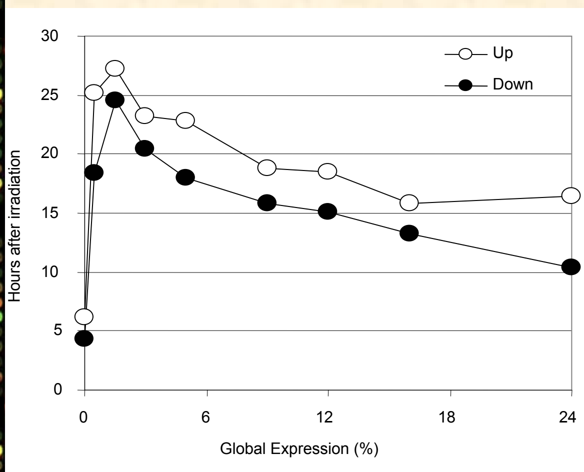
Gene Expression Profiling: Experimental Design

- **Recovery of *D. radiodurans* (wild-type strain R1) from acute radiation (exposure dose = 15,000 Gy of γ-radiation)**

Cell Sample	Recovery Time (in hours) @ 32°C
Control (non-irradiated)	–
1	0
2	0.5
3	1.5
4	3
5	5
6	9
7	12
8	16
9	24

Number of biological replicates (different mRNAs): 3

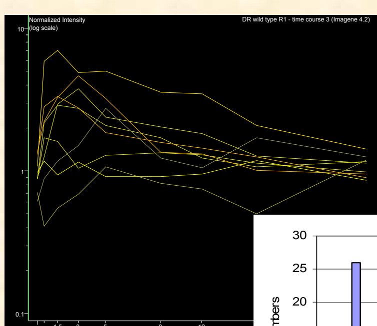
Percentages of the genes induced after radiation



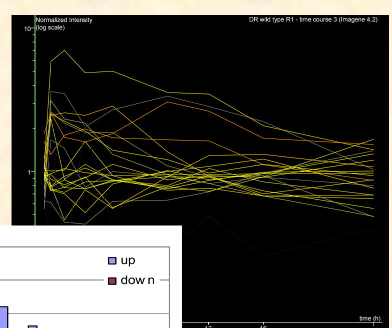
- More than 50% genes are induced at 1.5 hr radiation.
- More genes are up-regulated than down-regulated.

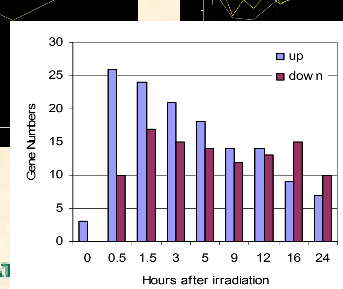
Genes involved in DNA Repair are highly induced

SOS Repair (RecA/LexA)



Recombinational Repair





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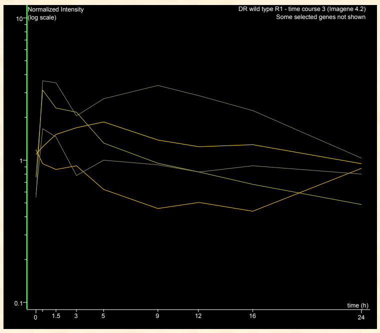
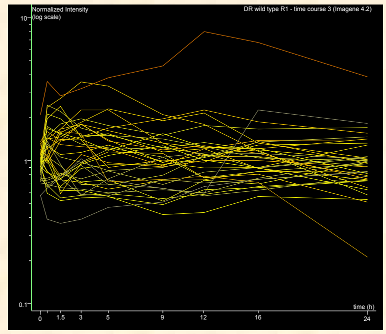
Genes in transcription are highly induced

RNA Polymerases

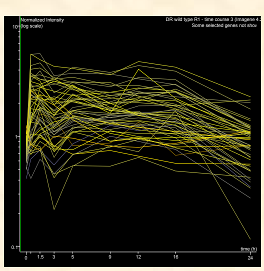


Transcription Regulators

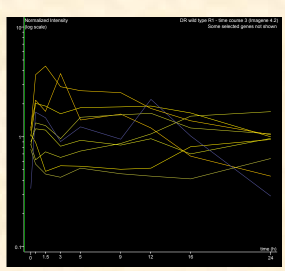


Genes involved in translation are highly induced

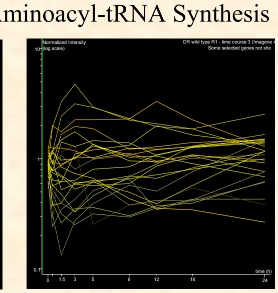
Ribosomes



Translation Factors

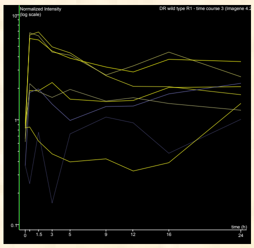


Aminoacyl-tRNA Synthesis

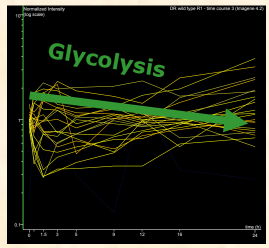


Genes involved in energy metabolism are highly induced

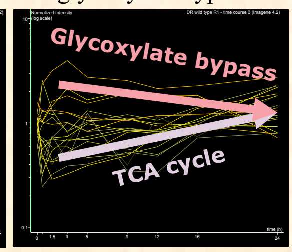
ATP Synthase



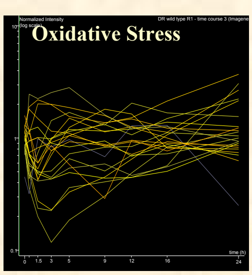
Glycolysis

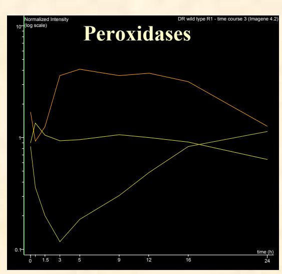


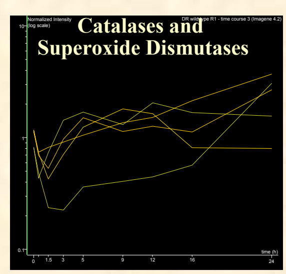
TCA Cycle-glycoxylate bypass



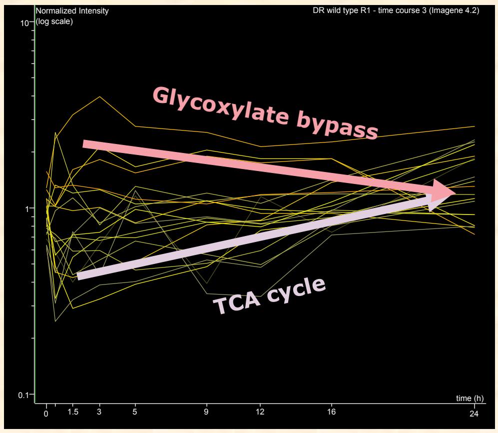
Genes involved in stress responses are highly induced to remove free radicals







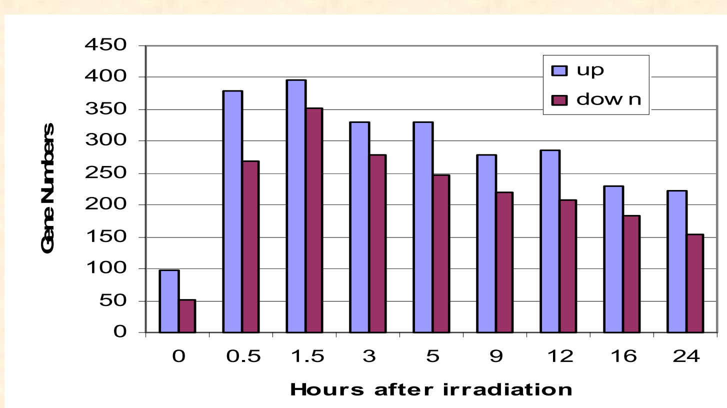
Energy pathway switch to minimize Free Radicals



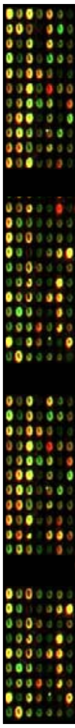
- TCA cycle produces more energy as well as more free radicals.
- Glycoxylate bypass produce less energy but no free radicals.

Hypothetical Proteins

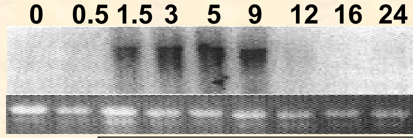
- **More than 40% genes whose expression is significantly changed upon irradiation encode hypothetical proteins.**



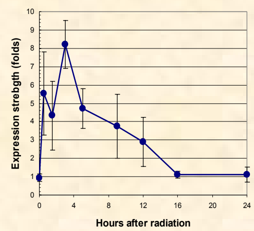
recA Gene Expression Profile

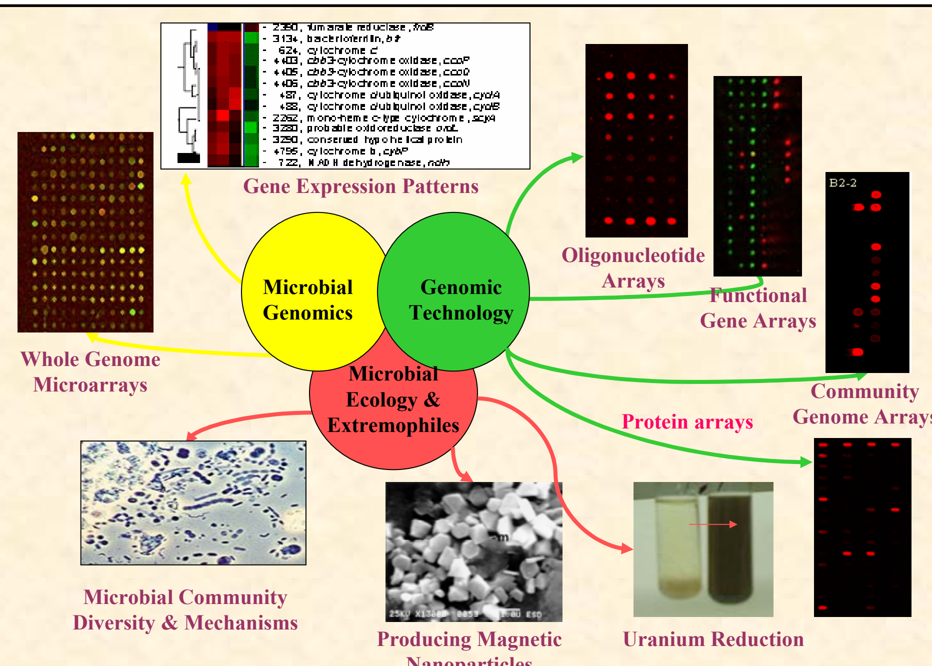


1. Northern analysis
From Carroll et al. J. Bacteriol. 178: 130-135 (1996)



2. Microarray analysis





Acknowledgement

(1)

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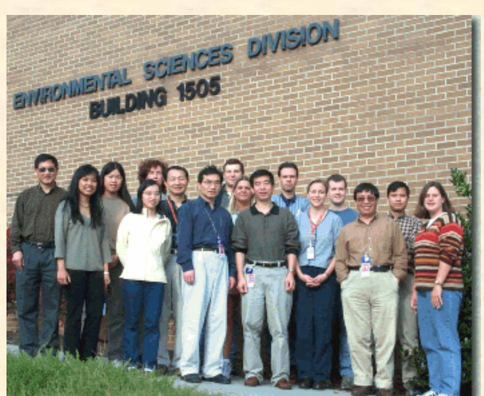
Microbial Genomics Group



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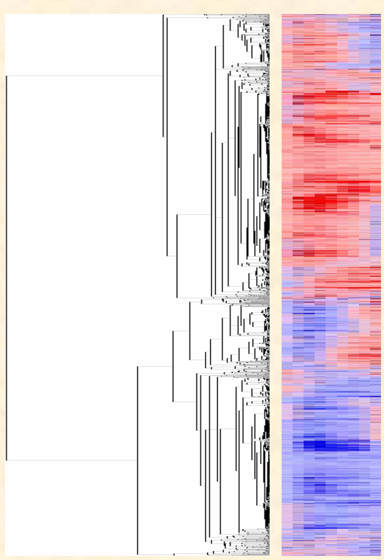
Genes which highly induced



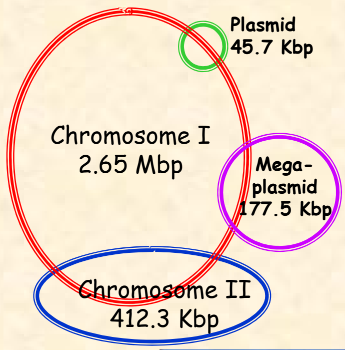
DNA repair
Transportation

Stress responses
Energy metabolism
Protein synthesis
DNA repair
Energy metabolism
Other cellular activities
More hypothetical proteins

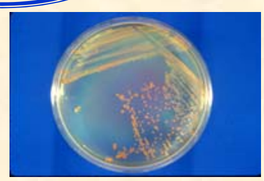
Amino acid
Nucleotide
Fatty acid

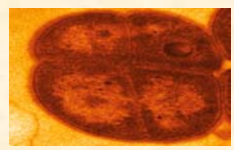
Amino acid
Nucleotide
Fatty acid
DNA synthesis

Deinococcus radiodurans R1 Genome: 3.3Mb



% G+C	66.6%
# ORFs	3,195
Mean ORF size	937 bp
% Coding	91%
# Similar to known proteins	52.2%
# Conserved hypothetical	16%
# Hypothetical	31.5%
rRNA operons	9





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